

Alterações fisiológicas decorrentes da interação organismo animal (ave) e microbioma do trato intestinal

“Interação extensa ocorre entre a ave (animal) hospedeira e seu microbioma do intestino. Estas interações estão manifestadas particularmente na troca de nutrientes, modulação da morfologia, fisiologia e imunidade do hospedeiro.

A maior parte dos carboidratos dietéticos facilmente digestíveis são digeridos e absorvidos pelo hospedeiro na secção proximal do intestino, deixando carboidratos não-digestíveis e -digeridos (residual dos carboidratos digestíveis) para bactérias residentes na parte distal do intestino. Muitas bactérias podem hidrolizar polissacarídeos, oligossacarídeos e dissacarídeos indigestíveis em açúcares que os compõe, que podem então ser fermentados pelas bactérias intestinais, produzindo ácidos graxos de cadeia curta (AGCCs), ou também denominados ácidos graxos voláteis (AGVs), principalmente acetato, propionato e butirato (mas também lactato, por exemplo). Os AGCCs podem ser utilizados pelo hospedeiro como energia e fonte de carbono. Tal fermentação pode ser observado na maior parte do trato intestinal de aves (do papo ao ceco), mas ocorre principalmente no ceco, que é densamente populado com bactérias.... AGCCs, especialmente butirato, pode servir como uma importante fonte de energia para células epiteliais intestinais. Em adição, tem sido relatado que AGCCs podem regular o fluxo sanguíneo, estimular crescimento e proliferação dos enterócitos, regulação da produção de mucina, e afeta respostas imunes intestinais.

Bactérias intestinais também afetam o metabolismo de nitrogênio do hospedeiro. ...

O período pós-eclosão é um estágio crucial para o crescimento e saúde das aves na transição que o animal faz da sua fonte de nutrientes entre a gema para dietas baseadas em carboidratos e proteínas. Para acomodar esta transição rápida da fonte de nutrientes, os órgãos digestórios do recém-nascido passam por mudanças anatômicas e fisiológicas e são os órgãos de desenvolvimento mais rápido durante o período pós-eclosão (perinatal). O trato intestinal em franco desenvolvimento providencia uma nicho ideal para colonização microbiana. Nesse mesmo tempo, o microbioma intestinal também desempenha um papel importante no desenvolvimento intestinal. ...

O microbioma intestinal pode também afetar a morfologia intestinal de aves. Os vilos intestinais são mais curtos e as criptas são mais rasas em aves livres de germes ou aves colonizadas por uma carga baixa de bactérias comparadas com aves criadas de forma convencional. ...” Tradução modificada de Pan e Yu, 2014 (palavras sublinhadas foram adições ao texto).

Referências para caso sobre fisiologia do trato intestinal, proteção e crescimento em frangos

- Alteração do crescimento da mucosa por AGCC (AGVs) – “assinatura” da presença de fermentação microbiana no trato intestinal

Kripke SA, Fox AD, Berman JM, Settle RG, Rombeau JL. 1989. Stimulation of intestinal mucosal growth with intracolonic infusion of short-chain fatty acids. J. Parenter. Enter. Nutr. 13:109–16

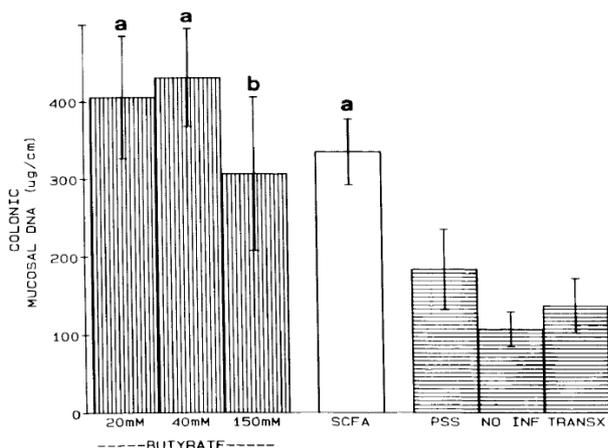


FIG. 2. Effect of 7 days of intracolonic butyrate or SCFA infusion on colonic mucosal DNA ($\mu\text{g}/\text{cm}$). 20 mM, 40 mM, 150 mM = butyrate infusions; SCFA = infusion of 70 mM acetate + 35 mM propionate + 20 mM butyrate; PSS = saline infusion; NO INF = no infusion; TRANSX = transection and reanastomosis of proximal colon without cecectomy or infusion. a, $p < 0.05$ vs PSS, NO INF, TRANSX; b, $p < 0.05$ vs NO INF, TRANSX.

Infusões no colon de ratos alimentados com dieta sem fibras e com transecção do ceco (retirada do ceco) e religação entre íleo e colon; Diferentes concentrações de butirato na infusão, ou SCFA = Ácidos graxos de cadeia curta (70mM acetato, 35 mM propionato e 20 mM butirato), ou ainda PSS = solução salina, NO INF = sem infusão e TRANSX = cirurgia de retirada do ceco e reinplantação do ceco.

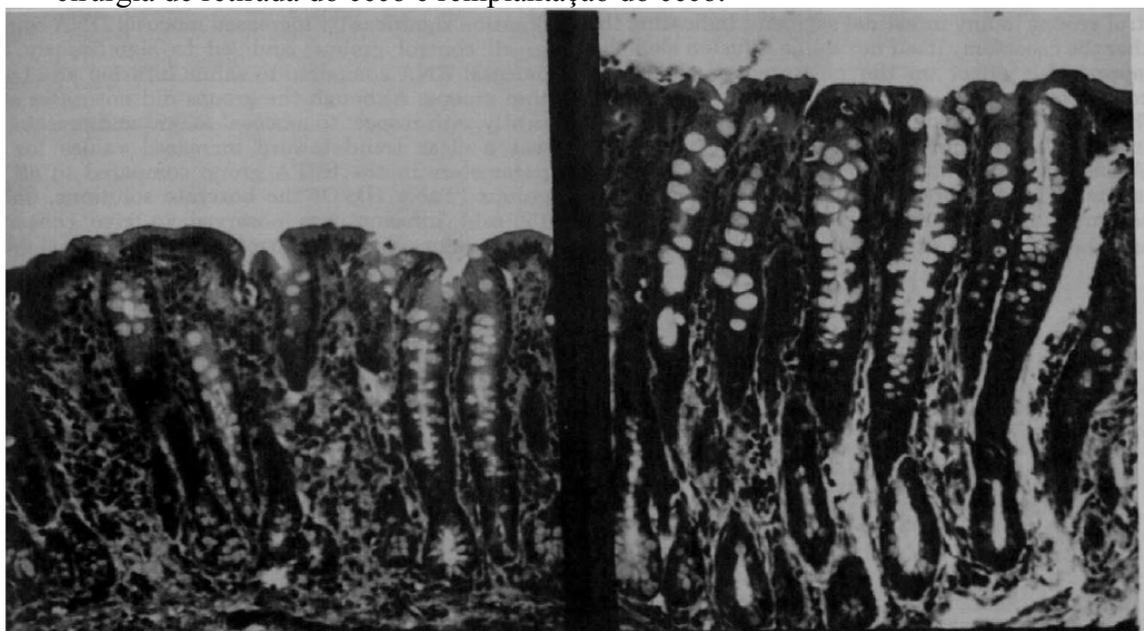


FIG. 3. Light micrographs of representative cross-sections of proximal colons from rats receiving intracolonic saline infusion (left) and 20 mM

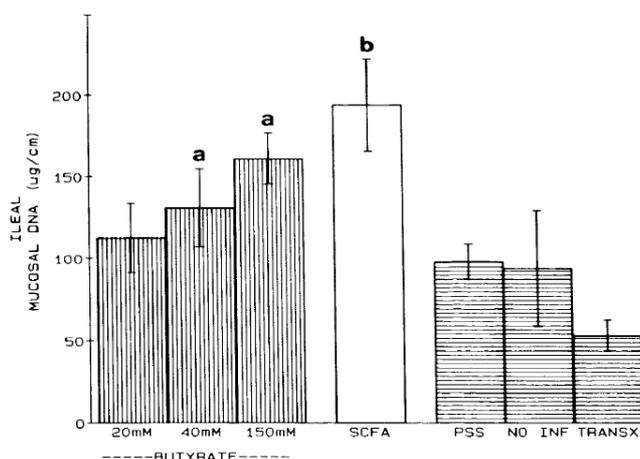


FIG. 4. Effect of 7 days of intracolonic butyrate or SCFA infusion on ileal mucosal DNA ($\mu\text{g}/\text{cm}$). a, $p < 0.05$ vs TRANSX; b, $p < 0.05$ vs PSS, NO INF, TRANSX, 20 mM. (see Fig. 2 for group definitions.)

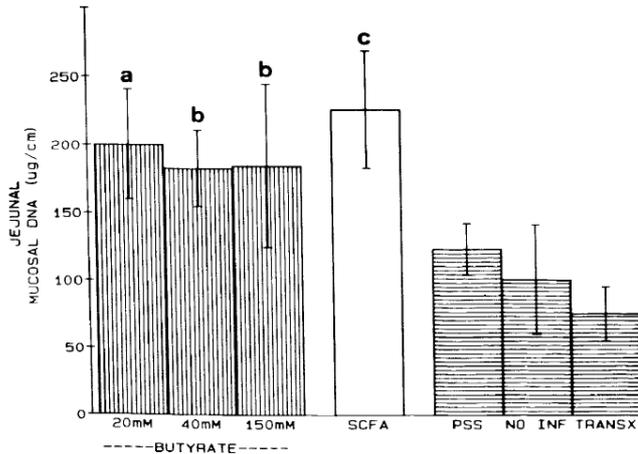


FIG. 5. Effect of 7 days of intracolonic butyrate or SCFA infusion or jejunal mucosal DNA ($\mu\text{g}/\text{cm}$). a, $p < 0.05$ vs NO INF, TRANSX; b, $p < 0.05$ vs TRANSX; c, $p < 0.05$ vs PSS, NO INF, TRANSX. (See Fig. 2 for group definitions.)

- Maior fluxo sanguíneo para o trato intestinal por estímulo de AGGC

Mortensen FV, Nielsen H, Mulvany MJ, Hessov I. 1990. Short chain fatty acids dilate isolated human colonic resistance arteries. *Gut* 31:1391-94

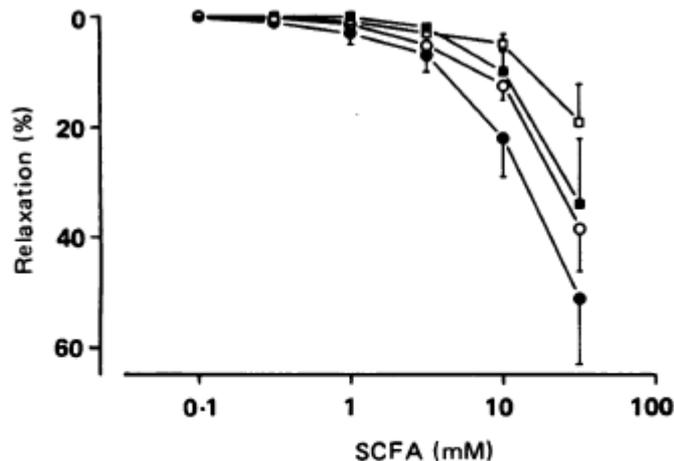


Figure 2: Effects of short chain fatty acids (SCFA) on human colonic resistance arteries already contracted with 50 mM KCl: (○) acetate; (■) propionate; (□) butyrate. The solid circles (●) show the concentration of acetate in mixture with propionate and butyrate ((acetate):(propionate):(butyrate)=60:35:25). Note the logarithmic division of the abscissa. Values are mean (SEM) (eight arteries).

- Maior recrutamento de neutrófilos (heterófilos em aves) e linfócitos pela atuação de butirato

Sanderson IR. Short chain fatty acid regulation of signaling genes expressed by the intestinal epithelium. *J Nutr* 2004; 134:2450S-4S

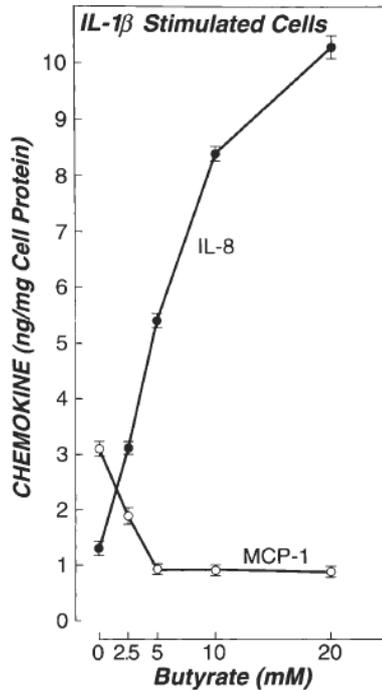


FIGURE 3 Effect of IL-1 β and butyrate on IL-8 and MCP-1 secretion by Caco-2 cells. IL-1 β alone stimulated the secretion of both

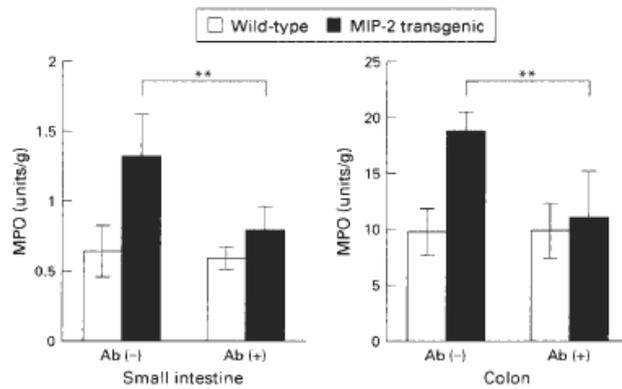


FIGURE 2 Neutrophil recruitment is increased in expressing MIP-2 in the intestinal epithelium. These effects were blocked with a MIP-2 antibody. Data are means \pm SD of 8 animals for each group. ** P < 0.01. Reproduced from (4) with permission

Figura 3. Aumento da quimiocina IL 8 pelo incremento concentração de butirato por células intestinais estimuladas pelo agente pró-inflamatório IL-1 β ; Figura 2 – Resposta de recrutamento de neutrófilos (medida pela atividade de MPO – mieloperoxidase – enzima importante para destruição de bactérias pelos neutrófilos) em diferentes segmentos do trato intestinal de ratos preparados para resposta na região promotora de expressão de IL 8 (MIP -2 transgenic). A maior expressão de MPO é entendida como neutrófilos presentes nos tecidos (atraídos = recrutados) por maior expressão de quimiocinas.

- Impacto do muco Mucina na proteção contra virulência de bactéria intestinal

Alemka A, Whelan S, Gough R, Clyne M, Gallagher ME, Carrington SD, Bourke B. Purified chicken intestinal mucin attenuates Campylobacter jejuni pathogenicity in vitro. J Med Microbiol 2010; 59:898-903

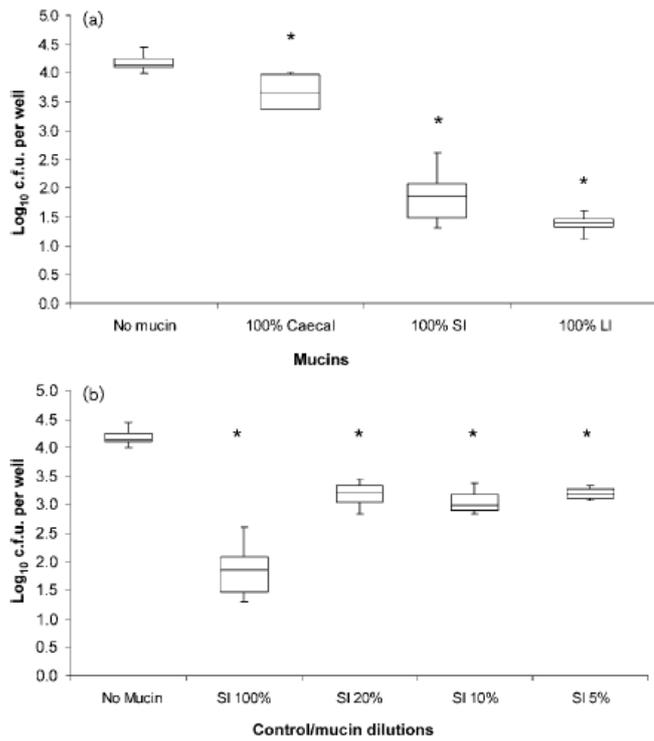


Fig. 2. Chicken mucin inhibits *C. jejuni* internalization into HCT-8 cells. (a) *C. jejuni* was treated with purified undiluted small intestinal (SI), large intestinal (LI) and caecal mucin prior to infecting HCT-8 cells. *Significant difference compared to control *C. jejuni* that was not treated with mucin. Caecal $P < 0.05$, SI $P < 0.005$, LI $P < 0.005$. (b) Effect of dilutions of purified small intestinal chicken mucin on *C. jejuni* invasion of HCT-8 cells. *Significant difference compared to control *C. jejuni* that was not pre-treated with chicken mucin. SI $P < 0.005$, 20% SI $P < 0.005$, 10% SI $P < 0.05$, 5% SI $P < 0.05$. 100% mucin represents 1 mg ml^{-1} .

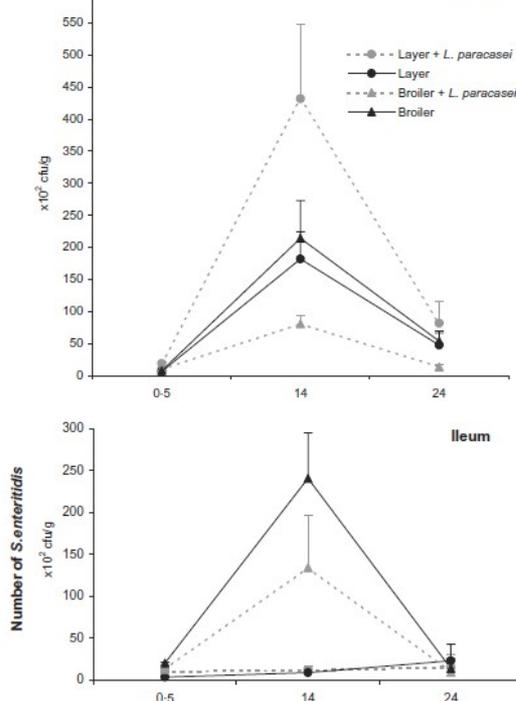
- Anticorpos e a defesa contra invasão por Salmonela

Koenen ME, Kramer J, van der Hulst R, Heres L, Jeurissen SH, Boersma WJ. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. *Br Poult Sci* 2004

Table 2. Total serum IgM and IgG titres (SEM) in meat-type strains ($n = 15$) at d 7 after priming and booster immunisation with TNP-KLH and continuous feeding of fermented liquid feed containing probiotics

		IgM titre	IgG titre
Priming	Standard sample	5698.2 (69.2)	1079.8 (15.9)
	Control	2320.7 (36.9) ^a	1303.7 (29.4) ^a
	<i>L. paracasei</i> + <i>L. plantarum</i>	3671.2 (59.2) ^c	2261.7 (71.3) ^b
	<i>L. plantarum</i>	3692.1 (102.2) ^{b/c}	1789.7 (63.0)
Boost	Control	3924.3 (62.6) ^a	4394 (67.2) ^a
	<i>L. paracasei</i> + <i>L. plantarum</i>	5378.2 (83.8) ^b	6800.5 (127.9) ^{c/d}
	<i>L. plantarum</i>	7899.3 (169.3) ^{d/b}	3863 (34.0) ^a

^{a-c}Different letters in the same column stand for significant differences within the column (one letter difference $P < 0.1$; two letters difference $P < 0.05$; three letters difference $P < 0.01$; four letters difference $P < 0.001$).

Figure 1. *The modulation of the innate immune response by feeding L. paracasei LW 122 in layer- and meat-type strains.*

Aumento da fagocitose (número de bactérias) e ação bactericida na região do íleo e ceco da linhagem de corte, observadas às 14 horas após exposição *in vitro* à bactéria.

- Alteração de morfologia intestinal e atividade de enzimas digestivas associada com microflora por ação de dieta

Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. *Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers.* *Poult Sci* 2003; 82:1030-6

FRUCTOOLIGOSACCHARIDE ON INTESTINAL STRUCTURE AND FUNCTION

TABLE 2. Effect of FOS on growth performance of male broilers¹

Item	Dietary FOS ² level (g/kg)				SEM
	0	2.0	4.0	8.0	
ADG (g)	47.16 ^b	50.22 ^{ab}	52.53 ^a	49.41 ^{ab}	1.264
ADFI (g)	104.75 ^a	105.46 ^a	106.11 ^a	104.76 ^a	0.725
F/G	2.22 ^a	2.10 ^b	2.02 ^b	2.12 ^{ab}	0.035

^{a,b}Means within a row with different letters differ significantly ($P < 0.05$) when tested with Duncan's new multiple-range test following analysis of variance.

¹Data represent mean values of five replicate cages of 12 chicks each.

²FOS = fructooligosaccharide; ADG = average daily gain; ADFI = average daily feed intake; F/G = feed per gain.

TABLE 3. Viable cell counts of microflora in small intestinal and cecal digesta of male broilers^{1,2}

Site and microflora	Dietary FOS ³ level (g/kg)				SEM
	0	2.0	4.0	8.0	
Small intestine					
Total anaerobes	8.47 ^a	8.69 ^a	8.75 ^a	8.81 ^a	0.36
<i>Bifidobacterium</i>	7.22 ^b	7.82 ^{ab}	8.11 ^a	7.64 ^{ab}	0.25
<i>Lactobacillus</i>	7.46 ^b	8.11 ^{ab}	8.47 ^a	8.20 ^{ab}	0.31
<i>Escherichia coli</i>	7.03 ^b	6.46 ^{ab}	6.18 ^a	6.71 ^{ab}	0.22
Cecum					
Total anaerobes	9.55 ^b	9.73 ^{ab}	9.85 ^a	9.73 ^{ab}	0.09
<i>Bifidobacterium</i>	8.36 ^b	8.81 ^{ab}	8.94 ^a	8.68 ^{ab}	0.18
<i>Lactobacillus</i>	8.42 ^b	9.02 ^a	9.08 ^a	8.80 ^{ab}	0.15
<i>Escherichia coli</i>	7.72 ^a	7.11 ^b	7.17 ^b	7.73 ^a	0.17

^{a,b}Means within a row with different letters differ significantly ($P < 0.05$).

¹Bacterial numbers are expressed as log₁₀ colony-forming units per gram of DM.

²Means represent 15 chicks at 49 d of age (three chicks from each of five cages) per treatment for a total of five experimental units per treatment.

³Fructooligosaccharide.

TABLE 4. Effects of FOS on the digestive enzyme activities in the small intestinal contents of male broilers^{1,2}

Enzyme	Dietary FOS ³ level (g/kg)				SEM
	0	2.0	4.0	8.0	
Protease (unit)	65.97 ^b	75.53 ^{ab}	83.91 ^a	77.35 ^{ab}	5.104
Amylase (Somogyi unit)	8.42 ^c	12.80 ^{ab}	14.75 ^a	10.68 ^{bc}	0.956
Lipase (Sigma-Tietz unit)	22.68 ^a	24.37 ^a	20.91 ^a	25.86 ^a	2.754

^{a-c}Means within a row with different letters differ significantly ($P < 0.05$).

¹Amylase activity unit (1 Somogyi unit) was defined as the amount of amylase that would cause formation of reducing power equivalent to 1 mg of glucose in 30 min at 40°C/mg of intestinal digesta protein. Lipase activity unit (Sigma-Tietz units) was equal to the volume (mL) of 0.05 M NaOH required to neutralize the fatty acid liberated during 6 h incubation with 3 mL of lipase substrate at 37°C/mg of intestinal digesta protein. The protease activity unit was defined as mg of azocasein degraded during 2 h incubation at 38°C/mg of intestinal digesta protein.

²Means represent 15 chicks at 49 d of age (three chicks from each of five cages) per treatment for a total of five experimental units per treatment.

³Fructooligosaccharide.

TABLE 5. Effects of fructooligosacchride (FOS) on the morphology of the intestinal mucosa at different sites in the small intestine¹

Site	Dietary FOS level (g/kg)				SEM
	0	2.0	4.0	8.0	
Villus height (μm)					
Duodenum	674.13 ^a	652.71 ^a	688.16 ^a	670.33 ^a	20.26
Jejunum	780.45 ^a	803.61 ^a	814.73 ^a	803.04 ^a	28.27
Ileum	541.18 ^b	598.04 ^{ab}	625.40 ^a	570.13 ^{ab}	24.15
Crypt depth (μm)					
Duodenum	481.52 ^a	487.09 ^a	498.67 ^a	482.25 ^a	22.14
Jejunum	530.92 ^b	495.51 ^{ab}	441.16 ^a	499.27 ^{ab}	26.75
Ileum	436.44 ^b	367.81 ^{ab}	325.33 ^a	389.35 ^{ab}	30.81
Villus height: crypt depth					
Duodenum	1.40 ^a	1.34 ^a	1.38 ^a	1.39 ^a	0.06
Jejunum	1.47 ^b	1.62 ^{ab}	1.85 ^a	1.61 ^{ab}	0.09
Ileum	1.24 ^c	1.63 ^b	1.92 ^a	1.46 ^{bc}	0.08

^{a-c}Means within a row with different letters differ significantly ($P < 0.05$).

¹Means represent 15 chicks at 49 d of age (three chicks from each of five cages) per treatment for a total of five experimental units per treatment. There was one sample for each of the three intestinal segments per chick, three cross-sections per sample, 45 cross-sections for each of the three intestinal segments per treatment, and 10 measurements per cross-section for a total of 450 measurements for each of the three intestinal segments per treatment).

TABLE 6. Effects of fructooligosaccharide (FOS) on the microvillus height at different sites in the small intestine¹

Site	Dietary FOS level (g/kg)				SEM
	0	2.0	4.0	8.0	
Duodenum (μm)	1.96 ^a	2.04 ^a	2.12 ^a	2.06 ^a	0.09
Jejunum (μm)	2.15 ^b	2.42 ^{ab}	2.61 ^a	2.43 ^{ab}	0.11
Ileum (μm)	1.40 ^c	1.75 ^{ab}	1.94 ^a	1.54 ^{bc}	0.08

^{a-c}Means in a row with different letters differ significantly ($P < 0.05$).

¹Means represent 15 chicks at 49 d of age (three chicks from each of five cages) per treatment for a total of five experimental units per treatment. There was one sample for each of the three intestinal segments per chick, three ultrathin sections per sample, 45 sections for each of the three intestinal segments per treatment, and 10 measurements per section for a total of 450 measurements for each of the three intestinal segments per treatment).